ANGIOCIDIN FRAGMENTS AND USES THEREOF IN CLINICAL ASSAYS FOR CANCER AND OTHER DISEASES

SPECIFICATION

1. FIELD OF INVENTION

The present invention relates to assays for detecting the presence of one or more modified forms or derivatives of angiocidin, particularly fragments and/or aggregates, as a diagnostic test for cancers and other diseases or conditions, the use of such fragments, aggregates and/or derivatives thereof as calibrators, competitors, and/or indicators in an assay, and to the fragments and aggregates themselves.

2. DESCRIPTION OF RELATED ART

Cancer is a cellular malignancy which causes the loss of normal control mechanisms and results in unregulated growth, lack of differentiation, and the ability to invade local tissues and metastasize. Thus, cancer cells are unlike normal cells, and are potentially identifiable not only by their phenotypic traits, but also by their biochemical and molecular biological characteristics and by biochemical and molecular biological changes they can induce in non-cancerous tissue, such as nearby stroma and endothelium. In particular, the altered phenotype of cancer cells indicates altered gene activity, which may be either unusual gene expression, or gene regulation. Identification of gene expression products or proteins, or modified proteins, such as fragments, that are associated with the presence of cancer cells will allow for the molecular detection, identification, and/or characterization of malignancies. The ability to diagnose suspected cancers, and to potentially identify not only cell type, but also predisposition for metastasis and any sensitivity to particular anti-cancer therapy, is useful for determining not only the course of treatment, but also the likelihood of success. Uses include, but are not limited to, screening an individual or group of individuals for the presence or development of a cancer; and following a patient with a known cancer for the response to or the effectiveness of therapy, including, for example, a recurrence.

Angiocidin, a cellular receptor, is over-expressed in invasive tumors and tumor microvessels (PCT application, WO 01/05968). Noninvasive tumors either do not express this receptor, or express it at very low levels (WO 01/05968).

Nevertheless, the prior art does not teach the existence of fragments or aggregates of angiocidin in tumor cells, and in fact, the prior art teaches against the existence of these or

other modified forms of angiocidin (e.g., p. 14 lines 2-6 of WO 01/05968 and Tuszynski GP et al. J Cell Biol. 1993 Jan;120(2):513-21 teaches "a single protein peak ... by CSVTCG affinity chromatography which also analyzed as a single peak by anion exchange chromatography" and as a single band on non-reducing SDS-PAGE gels). Nor is there any indication of their presence in a sample from a patient or subject, nor their value for a diagnostic technique (WO 01/05968). The present invention's concept of fragments and aggregates of angiocidin has been recently confirmed in human serum from cancer patients, in a public document (Sabherwal Y et al, 95th Annual Meeting of the American Association for Cancer Research, March 27-31, 2004, Orlando, Florida).

The present invention is an assay for fragments and/or aggregates of angiocidin, in distinction to an assay limited to angiocidin itself. The assay is for fragments and/or aggregates that are present in the blood, blood plasma, blood serum, and/or another bodily fluid or sample, such that an elevated concentration of a fragment and/or aggregate is taken as an indication that a mammal, particularly a human, has a cancer, a progression of a cancer, and/or a recurrence of a cancer. The assay can be combined with other cancer diagnostic or screening tests, including but not limited to an assay of PSA and/or an assay of angiocidin. This invention provides a basis for novel diagnostic assays that can be more specific, more sensitive, and/or more easily calibrated, than an assay solely of angiocidin itself, i.e., the intact, full-length molecule.

All references are incorporated herein by reference in their entireties. These references include, but are not limited to, PCT application WO 01/05968 and scientific publications.

BRIEF DESCRIPTION OF THE INVENTION

Important aspects of the invention are diagnostic methods and related kits that are based on the detection and quantification of angiocidin fragments and/or aggregates in bodily fluids, especially plasma or serum. Foremost among those diagnostic methods are those that detect or monitor the status of a cancer. Diagnostic methods that detect or monitor the status of another disease or condition are also contemplated, such as an immunologic condition, an infectious condition, an autoimmune condition, an allergic condition, an ischemic condition, a vascular condition, a condition associated with endothelial activation, an organ dysfunction, and organ failure, an inherited condition, and an acquired condition.

Aspects of the invention closely related to the diagnostic methods (assays) are

angiocidin fragment(s) that are detected in the plasma or serum, and angiocidin fragment(s) that can be used to induce and/or screen an antibody or other binding agent of interest for use in a diagnostic method or can be used in a competition-type or non-competitive diagnostic assay. It is understood that an antibody or other binding agent raised and/or selected for binding to an angiocidin fragment can be of particular use when it does, or does not, also bind to another angiocidin fragment, angiocidin, an angiocidin aggregate, and/or a modified form or derivative of angiocidin. As an illustrative example not meant to be restrictive, an antibody or other binding agent shown to bind to an angiocidin fragment and that also binds to angiocidin and to an angiocidin aggregate is useful in detection and quantification of total immunoreactive material in a sample, such as blood serum. As another illustrative example, an antibody shown not to bind to an angiocidin fragment, but to bind angiocidin, is useful, say, in combination with an assay of total immunoreactive material to determine a fragment level independent of angiocidin. Another important aspect of the invention includes the detection of angiocidin aggregates in cancerous versus non-cancerous plasma samples. Aspects of the invention also relate to methods of assaying proper sample collection by analysis of angiocidin fragments, aggregates and/or angiocidin itself. The sample includes but is not limited to blood, serum or plasma.

DETAILED DESCRIPTION OF THE INVENTION

This invention relates to the detection of angiocidin fragments and/or aggregates in the blood, plasma, serum, and/or another bodily fluid or sample from a patient for the purpose of a cancer diagnostic assay. The term "angiocidin" is used to refer not only to angiocidin itself but also to other molecules with essentially the same full-length sequences. These molecules include but are not limited to a proteasome 26S subunit, 26S proteasome non-ATPase regulatory subunit 4, anti-secretory factor-1, and S5a/antisecretory factor. These sequences can be found in WO 01/05968 or on the National Center for Biotechnology Database website at (http://www.ncbi.nlm.nih.gov/BLAST/Blast.cgi). For the purposes of this invention we will refer to these sequences generally as "angiocidin." Some of these sequences differ by the absence of a three-codon segment, i.e., nine base pairs, which can be readily seen on BLAST alignments. In addition, before its molecular identification as angiocidin, this molecule had been referred to as a thrombospondin receptor and as a CSVTCG receptor (see, for example, Tuszynski GP et al. J Cell Biol. 1993 Jan;120(2):513-21).

Angiocidin is a CSVTCG-specific tumor cell adhesion receptor, see patent application WO 0105968, also NCBI protein accession number CAC32386.1 and/or CAC32387.1 (corresponding to nucleotide accession numbers AX077201 and AX077202), that in humans has the following amino acid sequences, 380 and 377 amino acids in length, respectively:

CAC32386.1
mvlestmvcvdnseymrngdflptrlqaqqdavnivchsktrsnpennvglitlandcev
lttltpdtgrilsklhtvqpkgkitfctgirvahlalkhrqgknhkmriiafvgspvedn
ekdlvklakrlkkekvnvdiinfgeeevntekltafvntlngkdgtgshlvtvppgpsla
dalisspilageggamlglgasdfefgvdpsadpelalalrvsmeeqrqrqeeearraaa
asaaeagiattgtegerdsddallkmtisqqefgrtglpdlssmteeeqiayamqmslqg
aefgqaesadidassamdtsepakeeddydvxqdpeflqsvlenlpgvdpnneairnamg
slasqatkdgkkdkkeedkk (SEQ ID NO:1)

CAC32387.1
mvlestmvcvdnseymrngdflptrlqaqqdavnivchsktrsnpennvglitlandcev
lttltpdtgrilsklhtvqpkgkitfctgirvahlalkhrqgknhkmriiafvgspvedn
ekdlvklakrlkkekvnvdiinfgeeevntekltafvntlngkdgtgshlvtvppgpsla
dalisspilageggamlglgasdfefgvdpsadpelalalrvsmeeqrqrqeeearraaa
asaaeagiattgtedsddallkmtisqqefgrtglpdlssmteeeqiayamqmslqgaef
gqaesadidassamdtsepakeeddydvxqdpeflqsvlenlpgvdpnneairnamgsla
sqatkdgkkdkkeedkk (SEQ ID NO:2)

All assays for angiocidin fragments described herein are based on the presence of an epitope or other binding target. The assays will therefore detect not only an appropriate angiocidin fragment, but also the one that has been modified in a manner that did not destroy the epitope or other binding target. As such, detection of modified forms in the assays is consistent with the intention of the present invention. Identification and/or quantification of said epitope through a method not requiring a binding agent is also contemplated, including but not limited to sequencing and/or mass spectrometry.

For all inventions specified herein, reference to angiocidin is intended to encompass both the form with the amino acid sequence, SEQ ID NO:1, and that with the amino acid sequence, SEQ ID NO:2. As defined in WO 0105968, and for the purposes of the present application, these amino acid sequences in their entirety and in monomeric form are considered to be angiocidin, meaning the full-length, intact, unmodified molecule. It is further appreciated that angiocidin, as defined by SEQ ID NOs: 1 and 2, may contain a signal sequence, transmembrane sequence, cell-anchoring sequence, cell-attachment sequence, or other domain that is subsequently removed by cleavage or digestion, thereby

yielding one or more new molecules that are no longer angiocidin, but angiocidin fragments. It is also understood that angiocidin and/or its fragments may be modified in other ways, such as covalently or non-covalently, and/or by forming homo- or hetero-dimers or higher molecular weight aggregates that are likewise no longer angiocidin. Minor genetic or person-to-person variations in the sequence may of course occur. Such sequence variations are to be distinguished from fragmentation, which implies complete elimination and/or separation of sequences at the amino and/or carboxyl terminus and/or the middle of angiocidin.

Angiocidin may be derived from cancer tissues, such as melanoma cells or lung carcinoma cells. Analysis of by sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) of material from mammalian tumor cells or cell lines has shown that angiocidin has an apparent molecular weight of 50 kD under non-reducing conditions (See WO 01/05968). As used herein, the term "non-reducing conditions" refers to conditions under which the material is not reacted with a reducing agent, such as mercaptoethanol. In some of those preparations, small amounts of material were reported with molecular weights of greater than 100 kD (WO 01/05968), but without any indication of their presence in a bodily fluid, nor their value for a diagnostic technique. Conversely, reducing conditions refers to conditions wherein the material to be analyzed is reacted with a reducing agent prior to SDS-PAGE electrophoresis. After exposure to reducing conditions, it was reported that the protein migrates as two major polypeptide bands spaced closely together with apparent molecular weights of 50 and 60 kD, "consistent with the interpretation that the protein [angiocidin] ... assume[s] a more compact configuration when disulfide bonded." (quote from WO 01/05968). Angiocidin is classified as a glycoprotein since purified material binds galactose-, mannose-, and glucosamine-specific lectins. It does not cross-react with antibodies against integrins, laminin, or CD36 (International PCT application, WO 01/05968).

Recent data by Sabherwal et al. shows an angiocidin Western blot of sera from cancer patients compared to normal controls (Saberwhal et al., 2004). No fragments or aggregates were evident in any normal control sample. The results show that an angiocidin fragment or fragments of lower molecular weight than angiocidin are present in sera from subjects with colon carcinoma with liver metastasis and from a subject with breast cancer. Angiocidin aggregates (and/or aggregates of an angiocidin fragment) of a higher molecular weight than angiocidin are also present in one of the colon carcinoma serum samples as well

as in the breast carcinoma serum sample and in one of the serum samples from a patient with rectal carcinoma. The results also show material apparently co-migrating with angiocidin, which may be a molecule that comprises SEQ ID NO:1 or 2, although alternatively, it may be missing short sequences, in which case it would not be angiocidin. In at least two of the serum samples (one from breast cancer and one from rectal cancer), the purported angiocidin band is not markedly darker than in a normal sample (or in the case of one of the samples from a rectal cancer patient, angiocidin is absent), confirming the current invention's conception of the importance of detecting and/or quantifying fragments and/or aggregates. Thus, one embodiment of the present invention is to find antibodies or other binding agents that detect fragments, as well as angiocidin and aggregates, for use in an ELISA or ELISA-like assay or other assays well-known in the art. A second Western blot shown by Sabherwal et al. (2004) shows increasing signals in serum samples from individuals with increasingly advanced stages of breast cancer, although the stained band that is purported to be angiocidin migrates with decreasing apparent molecular weight in more advanced stages, consistent with a modified form.

To perform an SDS-PAGE, the material or sample to be analyzed is solubilized in a solution of sodium dodecyl sulfate (SDS), and then electrophorsed through an SDS-gel, for example an SDS gel of 8% polyacrylamide. SDS-PAGE is well-known in the art, and illustrative protocols are found, for example, in U.S. Application No. 10/419,462, Pan M et al. J Clin Invest 113:1277-1287, 2004, and Tuszynski GP et al. J Cell Biol. 1993 Jan;120(2):513-21. Molecular markers (typically proteins) of known molecular weight are run on the same or parallel gels. To identify and distinguish angiocidin fragments, angiocidin, and angiocidin aggregates, preferred markers migrate at molecular weights similar to angiocidin (~50kDa), below angiocidin (i.e., lower apparent molecular weight, which is helpful to assess fragments), and above (i.e., higher apparent molecular weight, which is helpful to assess aggregates). Illustrative molecular weight markers are at 184 kDa, 121 kDa, 86 kDa, 67 kDa, 52 kDa, 40 kDa, 28 kDa, and 22 kDa. These and similar molecular weight markers are commercially available from a variety of sources, such as Invitrogen Life Technologies (http://www.invitrogen.com/). One illustrative example from Invitrogen is the "MagicMark" molecular weight standards for Western blots, in which each of eight protein bands, ranging in size from 20-120 kDa, is a recombinant protein, with the size variation due to repetitive units of a protein fused to an IgG binding sequence. Molecular weight standards are also available from Biorad (http://www.biorad.com/),

including "Precision Plus" recombinant proteins (250, 150, 100, 75, 50, 37, 25, 20, 15, and 10 kDa), as well as non-recombinant protein molecular weight standards (e.g., Kalaidoscope Prestained Standards: myosin at 216 kDa, β-galactosidase at 132 kDa, bovine serum albumin at 78 kDa, carbonic anhydrase at 45.7 kDa, soybean trypsin inhibitor at 32.5 kDa, lysozyme at 18.4 kDa, and aprotinin at 7.6 kDa). The electrophoresed material at various positions in the gel can be visualized by protein stain (e.g., Coomassie blue stain), by Western blot analysis, or other technique. As stated, for angiocidin the peak fraction when tumor cell extract was passed over a CSVTCG column was analyzed on SDS-gel electrophoresis under nonreducing conditions and revealed a single major band with an apparent molecular weight of 50 kD. Under reducing conditions (5% beta-mercaptoethanol), the same material appeared as two polypeptide bands of 50 and 60 kD. (See WO 01/05968). Other methods well-known in the art, such as mass spectrometry, are also available to assess molecular weight. To identify and distinguish angiocidin fragments, angiocidin, and angiocidin aggregates, sequencing technologies can also be used. As an illustrative example not meant to be restrictive, sequencing technology is used to identify an N-terminal sequence and/or a C-terminal sequence of a molecule from a bodily sample of interest. Said sequence is then compared with SEQ ID: NO 1 and/or SEQ ID: NO2, for example, to determine if the molecule is angiocidin or is missing a portion.

The following protocol (specifically noted for a plasma sample) can be used for the SDS-PAGE electrophoresis:

I. Sample Preparation

Protease inhibitors added:

1μl of leupeptin solution (1 mg/ml in sterile water) is added per ml plasma 10μl of PMSF solution (1.74 mg/ml in isopropanol) is added per ml plasma.

4x sample buffer:

dH_2O 4.0 ml; 0.5M tris-HCl 1.0 ml; glycerol 0.8 ml; 10% SDS 1.6 ml; β-mercaptoethanol 0.4 ml; 0.05 % bromophenol blue 0.2 ml (for non-reducing conditions, β-mercaptoethanol is omitted)

5μl plasma samples are diluted with 20μl distilled water, and 25μl 2x sample buffer is added, followed by heating (to aid disulfide bond reduction under reducing conditions, and

to aid solubilization under reducing or non-reducing conditions). 10µl of each sample mixture is then run on the gel.

In an example of an alternative to the Standard Gel Electrophoresis Procedure, to aid reduction and denaturation, blood plasma is mixed with 5% fresh mercaptoethanol and 4-6 M fresh urea and boiled for at least 5 minutes in a fume hood.

Alternatives to the plasma sample include but are not limited to serum or another bodily fluid or a biopsy sample can be used. Clearly in some situations, prior to the SDS-PAGE procedure, a sample of bodily fluid or tissue is subjected to a fractionation or purification procedure before electrophoresis, including but not limited to dialysis, chromatography, size chromatography, affinity chromatography, affinity chromatography using a CSVTCG column, immunoaffinity chromatography, adsorption, immunoadsorption, isoelectric focusing, centrifugation, sedimentation, floatation, precipitation, immunoprecipitation, extraction, and gel filtration.

II. Electrophoresis

Gel electrophoresis is done on SDS-polyacrylamide gels (4% stacking, 10% running gel) in tris/glycine/SDS buffer (see running buffer below, pH 8.3) at 200 V/7-8 cm at 25°C for 34 minutes. Alternative electrophoretic set-ups and procedures are well-known in the art and can be used (e.g., using gels of about 8%-12% acrylamide; omission of the stacking gel), but should reliably separate angiocidin (50-60 kDa) from aggregates and fragments, as noted above. Separation of the appropriate molecular weight standards should also be achieved, as noted above.

5x running buffer pH 8.3: Tris Base 15 g; Glycine 72 g; SDS 5 g; distilled water to 1 liter

(End of protocol)

Healthy adult tissues typically do not express angiocidin, or express it at low levels, whereas invasive tumors express it at high levels. Pre-invasive and/or pre-metastatic tumors also express angiocidin, but generally at lower levels than invasive tumors. As used herein, the term "level" refers to the magnitude of a quantity or concentration of a material, such as angiocidin or a fragment and/or aggregate, preferably considered in relation to a reference value. Angiocidin expressed in the context of a tumor is often modified in a way that causes

fragmentation, including but not limited to alternative splicing, cleavage by proteolytic digestion or hydrolysis, proteolytic digestion, hydrolysis, cleavage to separate a sequence segment before secretion by a cell, cleavage after secretion but before exit from a tumor cell (such as by local proteases), cleavage after exit from the tumor, cleavage in the bloodstream, and cleavage during sample collection or storage (such as by coagulation proteases that are activated when a blood sample clots during the preparation of blood serum). Various types of cleavage include but are not limited to cleavage of an N-terminal sequence, cleavage of a C-terminal sequence, cleavage of a signal peptide, removal of a signal peptide, cleavage of a targeting sequence, removal of a targeting sequence, cleavage of a region that mediates membrane association, cleavage of a region that mediates cell association, removal of a region that mediates association with a membrane or a cell, a proteolytic cleavage, a non-proteolytic cleavage, a hydrolytic cleavage, and an oxidative cleavage. Angiocidin expressed in the context of a tumor is often modified in a way that causes aggregation, including but not limited to self-aggregation, aggregation with another molecule, aggregation of an angiocidin fragment with another angiocidin fragment, aggregation of an angiocidin fragment with a fragment of a molecule that is not angiocidin, aggregation of a fragment with a molecule that is not a fragment, sulfhydryl linkage with a second molecule or fragment, covalent linkage with another molecule or moiety, ionic linkage with another molecule or moiety, hydrophobic association with another molecule or moiety, homodimerization, heterodimerization, formation of higher order complexes, a modification that exposes a neoepitope, a modification that preserves a native epitope, aggregation within the tumor, aggregation after exit from the tumor, aggregation in the bloodstream, and aggregation during sample collection or storage.

This invention contemplates the use of angiocidin fragments as a method of detecting, diagnosing, and/or following the course of a cancer. Specifically, levels (e.g., concentrations or amounts) of angiocidin fragments can be measured in an organism's blood, blood plasma, serum, biopsy, or other tissue or fluid. The level may be normalized in some appropriate fashion, including but not limited to the volume of the sample, the weight of the sample, the total amount of protein in the sample, and a value or values in a reference or control sample such as from healthy individuals. For a sample that contains cells, total DNA or nucleic acid can be used, as well as housekeeping genes or their products, which are well-known in the art and include but are not limited to actin, cyclophilin, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), 18S, and total protein.

In one embodiment, the organism is a mammal, including but not limited to a domesticated animal, pet, a companion animal, a porcine, equine, canine, feline, bovine or mouse. In a preferred embodiment, the mammal is human. The invention also contemplates that other molecules associated with angiocidin can also be fragmented and otherwise modified, and that these fragments or modified forms of angiocidin and others are useful in diagnostics, just as the angiocidin fragments are indicated herein.

Also contemplated is a fragment or fragments generated as incomplete sequences (illustrative mechanisms include, but are not limited to, alternative splicing and/or mRNA editing). Also contemplated is a combination or combinations of processes to generate a fragment (an illustrative but not limiting example would be alternative splicing to generate an incomplete protein, followed by cleavage to remove a cell-anchoring sequence). It is understood that secretion versus release via other processes may generate different patterns of angiocidin fragments. As an illustrative but not restrictive example, immunoreactive material that is secreted from a cell should be missing a signal peptide and/or a cell-anchoring sequence. Immunoreactive material that is released from a cell via cell lysis will not necessarily have a signal peptide or a cell-anchoring sequence removed. Cell lysis may subject angiocidin or its fragments to lytic processes other than the specific removal of a signal peptide or a cell-attachment sequence. Therefore, the level of an angiocidin fragment or fragments will be useful in indicating the patient's diagnosis or prognosis. Assay methods include but are not limited to those well-known in the art, such as ELISA, enzyme immunoassay (EIA), radioimmunoassay (RIA), Western blotting, immunohistochemistry, immunofluorescence, other immune-based methods, non-immune-based methods, quantitative methods, high through-put methods, automated methods, proteomic methods, a method using mass spectrometry, semi-quantitative methods and qualitative methods. It is understood that the art for mass specrometry includes protein and peptide sequencing, which is used to identify and even quantify these molecules.

The invention also contemplates using angiocidin aggregates as a method of detecting, diagnosing and/or following the course of a cancer. Angiocidin expressed in the context of a tumor may also be modified in a way that causes aggregation. The term "aggregation" may include but is not limited to self-aggregation, aggregation with another angiocidin molecule, aggregation of an angiocidin fragment with another angiocidin fragment, aggregation of an angiocidin fragment with a fragment of a molecule that is not angiocidin, and aggregation of a fragment with a molecule that is not a fragment.

Aggregation may be caused by sulfhydryl linkage with a second molecule or fragment, covalent linkage with another molecule or moiety, ionic linkage with another molecule or moiety, homodimerization, heterodimerization formation of higher order complexes, a modification that exposes a neoepitope, and a modification that preserves a native epitope.

In one embodiment, the diagnostic is used in order to detect a cancer. The cancer may be selected from the group consisting of an adenoma, adenocarcinoma, carcinoma, lymphoma, leukemia, solid cancer, liquid cancer, metastatic cancer, pre-metastatic cancer, non-metastatic cancer, a cancer with vascular invasion, internal cancer, skin cancer, cancer of the respiratory system, cancer of the circulatory system, cancer of the musculoskeletal system, cancer of a muscle, cancer of a bone, cancer of a joint, cancer of a tendon or ligament, cancer of the digestive system, cancer of the liver or biliary system, cancer of the pancreas, cancer of the head, cancer of the neck, cancer of the endocrine system, cancer of the reproductive system, cancer of the male reproductive system, cancer of the female reproductive system, cancer of the genitourinary system, cancer of a kidney, cancer of the urinary tract, cancer of a sensory system, cancer of the nervous system, cancer of a lymphoid organ, blood cancer, cancer of a gland, cancer of a mammary gland, cancer of a prostate gland, cancer of an endometrial tissue, cancer of a mesodermal tissue, cancer of an ectodermal tissue, cancer of an endodermal tissue, a teratoma, a poorly-differentiated cancer, a well-differentiated cancer, and a moderately differentiated cancer.

All assays described herein comprise the use of a binding agent that will bind to angiocidin fragments and/or aggregates. The agent can be an antibody or a non-antibody.

A binding agent can be selected from the group consisting of a polyclonal antibody, a monoclonal antibody, a single-chain antibody, a non-antibody, a protein, a product of phage display, an antibody or other binding agent to anti-secretory factor, an antibody or other binding agent to a fragment or aggregate of anti-secretory factor, an antibody or other binding agent to a proteasome 26S subunit, an antibody or other binding agent to a fragment or aggregate of a proteasome 26S subunit, an aptamer, a DNA, an RNA, a modified DNA, a modified RNA, a carbohydrate, a glycosaminoglycan, a heparin, a glycoprotein, a proteoglycan, and combinations and derivatives thereof. The binding agent may comprise a detectable label, wherein said label is either an intrinsic or added moiety, wherein the detectable label is selected from the group consisting of a radioactive label, a fluorescent label, a chemical label, a colorimetric label, an enzymatic label, a non-fluorescent label, a non-radioactive label, a biotin label, and an avidin moiety. In some applications, the

fragment or aggregate may comprise a detectable label, wherein said label is either an intrinsic or added moiety, wherein the detectable label is selected from the group consisting of a radioactive label, a fluorescent label, a chemical label, a colorimetric label, an enzymatic label, a non-fluorescent label, a non-radioactive label, a biotin label, and an avidin moiety.

Preferred binding agents are proteins, although non-proteins are also contemplated. Such proteins include both antibodies and nonantibodies.

Binding agents of the current invention can also be used for other well-known detection methods, including but not limited to immunohistochemistry and Western or ligand blotting.

In another aspect, any of several acceptable approaches can be used for the assay of an angiocidin fragment (or fragments), and/or aggregate (or aggregates) wherein the assay distinguishes the foregoing from angiocidin itself, and more than one of these approaches can, if desired, be used in a given assay. In one approach, the assay comprises a step wherein the fragment and/or aggregate is physically separated from the angiocidin. Generally that approach is combined with a step in which the presence of the fragment, aggregate, or angiocidin is shown by their reaction with a specific binding agent. Assay methods include but are not limited to those well-known in the art, such as ELISA, radioimmunoassay, Western blotting, immunohistochemistry, immunofluorescence, other immune-based methods, non-immune-based methods, quantitative methods, high throughput methods, automated methods, a method using mass spectrometry, semi-quantitative methods and qualitative methods. In particular embodiments, the physical separation technique is selected from the group consisting of dialysis, chromatography, size chromatography, affinity chromatography, affinity chromatography using a CSVTCG column, immunoaffinity chromatography, adsorption, immunoadsorption, isoelectric focusing, mass spectrometry, centrifugation, sedimentation, floatation, precipitation, immunoprecipitation, extraction, and gel filtration. In a preferred embodiment, the physical separation technique is gel electrophoresis.

In a second approach, the assay distinguishes a fragment (or fragments) based on one or more epitopes in angiocidin that are not present in said fragment. As an illustrative but not restrictive example, an epitope shared by angiocidin and an angiocidin fragment is used to obtain a quantitation of a total, angiocidin plus angiocidin fragment(s), from which is then subtracted a quantitation of angiocidin obtained using an epitope present in angiocidin but

not present in a fragment. The difference between the two quantitations is a quantitation of the amount of fragment. As an example, said quantitations can be performed using binding agents that recognize each of the said epitopes. Said binding agents are identified beforehand by assessing their ability to bind a fragment and/or angiocidin in a binding assay, such as a Western blot, a ligand blot, a Biacore binding assay, or an NMR-based binding assay. Binding agents (antibodies or other binding agents) that are specific for angiocidin can be identified by screening the binding agent against samples from a population of cancer patients and selecting a binding agent that is specific for angiocidin (as opposed to its fragments). As an illustrative example, said binding agent can be identified by reacting candidate binding agents with sera from individuals of a population of cancer patients, analyzing the binding agent reactive material on SDS-PAGE gels, and selecting a binding agent that, as evidenced by the gels, did not react with any fragments. Other methods to detect binding are also available, such as the ones listed above.

In a third approach, the assay distinguishes the fragment (or fragments) or aggregate based on one or more epitopes (here "epitope" meaning a target to which a binding agent, i.e., an antibody or a non-antibody, binds) in the fragment or aggregate that are not present in angiocidin, including but not limited to an epitope at an end of a fragment, an epitope that is displayed by a fragment but is shielded in angiocidin, and an epitope on an aggregate that is not detected in angiocidin.

PCT application WO 01/05968 shows that anti-angiocidin antibodies can be generated using native angiocidin as an immunogen and discloses that recombinant angiocidin would also be expected to work as an immunogen. Specificity of an antibody with regards to its target in the context of the present invention can be tested using a fragment or fragments of SEQ ID NO:1 or NO:2, or an aggregate of said fragment, fragments, or sequences. In a preferred embodiment, said fragment(s) or aggregate(s) are selected from the group consisting of an angiocidin fragment present in serum or plasma, an angiocidin fragment present in serum from an individual with a malignancy, an angiocidin fragment present in serum from an individual with a metastatic malignancy, and an angiocidin aggregate present in serum or plasma. Conversely, specific fragments of angiocidin, where the fragments are generated by recombinant techniques or by purification from serum or plasma, can be used to generate antibodies against specific regions, and therefore fragments, of the angiocidin molecule.

The assays and diagnostics of the instant invention are used to measure the level of

angiocidin fragments and/or aggregates in a bodily fluid, for example serum. The use of the term "and/or" is used to indicate that an assay can, if desired, be directed solely against a fragment (or fragments) or solely against aggregates. In measurements of plasma levels of fragments, aggregates, and/or angiocidin, it is preferred in some embodiments that the plasma is obtained by a method that prevents or reduces platelet activation and/or activation of a component of the clotting cascade during sample collection and/or storage; and/or by a method that prevents or reduces cleavage of angiocidin into fragments (or fragments into smaller fragments) during sample collection and/or storage. Platelet activation and/or activation of a component of the clotting cascade during sample collection and/or storage can result in the activation of proteases (including but not limited to a protease of the clotting cascade) that may cleave angiocidin and some angiocidin fragments, thereby complicating the assay. Formation of some aggregates may also be favored by clotting, and therefore would be preferred to avoid in some embodiments. To prevent or reduce platelet activation during sample collection and/or storage, the method may be one that does not comprise the use of a torniquet. In one embodiment, an assay of angiocidin is combined with a method to avoid activation of platelets and/or a component of the clotting cascade in a sample. Alternatively, an assay of immunoreactive material in a serum sample is performed, using a binding agent that recognizes a fragment, angiocidin, and an aggregate.

To prevent or reduce platelet activation and/or activation of clotting during sample collection and/or storage, the method may, for example, comprise a step selected from the group consisting of: (1) use of a large-bore needle, (2) discarding of the initial portion of the collected blood, (3) use of a coated needle, (4) use of a coated tubing, (5) storage of sample between -1°C and 5°C, and (6) separation of plasma within 30 minutes of sample collection. Also to prevent or reduce platelet activation and/or protease activity during sample collection and/or storage, the method may comprise the use of an agent selected from the group consisting of a platelet inhibitor, a protease inhibitor, a serine protease inhibitor, an enzyme inhibitor, an inhibitor of an enzyme that is divalent cation dependent, a heparin, a heparin fragment, a heparan, an anticoagulant, a COX inhibitor, an inhibitor of a cell-adhesion molecule, an inhibitor of a surface receptor, a glycoprotein inhibitor, an inhibitor of a glycoprotein IIb/IIIa receptor, a thrombin inhibitor, an inhibitor of degranulation, a chelator, a citrate compound, theophylline, adenosine, and dipyridamole (Diatube H vacutainers containing citrate, theophylline, adenosine, and dipyridamole are commercially available from Becton Dickinson; see Bergseth G et al. A novel enzyme immunoassay for

plasma thrombospondin: comparison with beta-thromboglobulin as platelet activation marker in vitro and in vivo. Thromb. Res. 99:41-50, 2000; such tubes can be referred to as CTAD tubes). Devices that minimize platelet activation and/or protease activity in a sample are also contemplated and include, but are not limited to, a collection tube containing a cocktail of platelet and/or clotting inhibitors, a collection tube containing a protease inhibitor, a collection tube containing an inhibitor of a protease that is or is derived from a blood component, and a device that discards or allows the easy discarding of the initial portion of collected blood. These methods can also be applied to samples of other body fluids.

A related aspect of the invention is a combination diagnostic test (especially for cancer) comprising at least two types of diagnostic tests, one of said tests being the assay for a angiocidin fragment (or fragments) and/or an aggregate in plasma, the other assay not being based on an angiocidin fragment and/or aggregate. Optionally, the other assay is one for angiocidin itself. In one set of embodiments, the test not based on a angiocidin fragment is selected from the group consisting of an imaging test, a radiographic test, a nuclear medicine test, a magnetic resonance imaging test, a blood test, a biopsy, a genetic test, a guaiac test, a test for fecal occult blood, and a test for fecal blood, a cancer test not based on a angiocidin fragment, a disease test not based on a angiocidin fragment, and an endoscopy. In particular embodiments of the foregoing methods, an angiocidin fragment comprises a detectable label (at least during some part of the method).

The blood test is selected from the group consisting of a cancer antigen test, a cancer gene test, a cancer DNA test, a cancer mRNA test, a cancer RNA test, a cancer protein test, a cancer glycoprotein test, a cancer carbohydrate test, a cancer lipid test, a prostate specific antigen test, a test of carcinoembryonic antigen, a test of cancer antigen CA-125, a test of alpha-fetoprotein, a test of CA15-3, a test of CA19-9, a test of malignin, a test of anti-malignin antibody, a test of anti-secretory factor, a cancer antigen that contains a carbohydrate epitope, a cancer antigen that contains a protein or polypeptide epitope, a cancer antigen that contains a lipid epitope, a cancer antigen that contains a mixed epitope, CA 27.29, episialin, and an angiocidin assay not based on an angiocidin fragment or aggregate. It is understood that some assays based on an angiocidin fragment and/or aggregate will broadly detect immunoreactive material, including angiocidin.

Detection can, for example, be part of a screening process. Such a screening could include a comparison against a reference value, involve a comparison against a previous

value from the same individual; and/or be done repeatedly and/or periodically (e.g., once a year, once every six months, or once every 2, 3, 4, 5 or 10 years.). It is understood that screening can be performed on humans and/or on non-human animals.

In another aspect, the invention includes a method to detect and/or quantify the angiocidin fragment or aggregate, said method comprising performing an immunoassay using an antibody. The immunoassay may be a Western Blot, an ELISA, a high throughput immunoassay, an automated immunoassay, a radioimmunoassay, immunohistochemistry, immunofluorescence, other immune-based methods, non-immune-based methods, quantitative methods, a method using mass spectrometry, high throughput methods, automated methods, and semi-quantitative methods and qualitative methods.

In a preferred embodiment, a recombinant angiocidin fragment is used as a standard for said assay or immunoassay. The recombinant angiocidin fragment is stable at 4°C, and does not contain hazardous, infectious agents. The recombinant fragment also does not vary from donor to donor, as it is not collected from a donor but rather produced in an organism such as bacteria. In a preferred embodiment, said fragment is selected to contain an epitope present in an angiocidin fragment and/or an epitope exposed on an angiocidin aggregate present in a bodily fluid, such as serum. Said fragment can be mixed or conjugated with another substance, such as a stabilizer. As an alternative, a synthetic fragment of angiocidin is used as a standard, including but not limited to a synthetic peptide fragment. In a preferred embodiment, said fragment is selected to contain an epitope present in an angiocidin fragment and/or an epitope exposed on an angiocidin aggregate present in a bodily fluid, such as serum. As another alternative, a fragment purified from serum is used as a standard.

In another aspect, the invention includes a method of distinguishing between a cancerous sample of bodily fluid and a non-cancerous sample of bodily fluid, said method comprising: 1) performing an assay on said samples using a binding agent; and 2) detecting angiocidin and/or angiocidin fragments and/or aggregates.

In another aspect, the invention includes a method to detect and/or quantify an angiocidin fragment or aggregate. In a preferred embodiment, the method distinguishes the angiocidin fragment or aggregate from angiocidin based on one or more epitopes in the fragment that are not present in angiocidin. In a further embodiment, the fragment or aggregate is distinguished based on one or more epitopes in angiocidin that are not present in the fragments or aggregates, said method comprising the steps of: 1) utilizing an epitope

shared by angiocidin and the angiocidin fragment or aggregate as a target for a binding molecule, such as an antibody, to obtain a quantitation of a total, angiocidin plus either the angiocidin fragment or aggregate, 2) utilizing an epitope present in angiocidin but not present in the fragment (and/or not in the aggregate) to obtain a quantitation of angiocidin only; and 3) utilizing the difference between the quantitations obtained in steps (1) and (2) as a quantitation of the amount of fragment or aggregate. In one embodiment, the method is applied to a sample of material taken or gathered from an organism. In a preferred embodiment, the organism is a human.

Many of the foregoing methods involve assays to detect an angiocidin fragment and/or aggregate of the invention. In each case, such fragments or aggregates can be referred to as "target" fragments for purposes of the assay. In many instances it is desirable to have the method also comprise a calibration step or procedure, in which known amounts of an angiocidin fragment (such as a peptide) are subjected to the method. Such "calibration" fragments are optionally detectably labeled. Labeling of a calibration fragment can be useful, for example, in independently quantifying their concentration. It is possible to perform the assays in which the target and calibration fragments comprise different detectable labels (or where one is detectably labeled and the other is not). In one embodiment of a competition assay also contemplated herein, a calibration fragment (as well as immunoreactive material in a sample) competes with a labeled target fragment, such that the greater the competition, the higher the measured level is.

It is understood that antibodies and other binding agents that recognize fragments normally also recognize angiocidin and aggregates. The converse is not necessarily true; e.g., fragmentation of angiocidin may result in a loss of epitope.

In specific embodiments of the invention, the angiocidin fragment or aggregate comprises a detectable label, wherein the detectable label is a target and/or indicator fragment and wherein a known or unknown amount of an unlabeled or differently labeled fragment is also subjected to the method, said unlabeled or differently labeled fragment also being an angiocidin fragment.

In another aspect, the invention includes a method of producing antibodies against an angiocidin fragment or aggregate, said method comprising administering said fragment or aggregate or immunogenic portion of said fragment or aggregate thereof to an organism capable of producing antibodies. Said fragment or aggregate or immunogenic portion of said fragment or aggregate can also be used to generate and/or select other binding agents,

including but not limited to non-antibodies.

Conventional antibodies are also referred to herein simply as "antibodies" as opposed to "single chain antibodies." An example of a conventional antibody is IgG, which is composed of two heavy chains and two light chains. Raising conventional antibodies is merely one of a number of methods that are generally based on the approach of random, semi-random, directed, combinatorial, and/or other means for the generation of large numbers of diverse peptides and/or non-peptides, that is then followed by a selection procedure to identify within this large number those peptides and/or non-peptides that bind to a target and/or an epitope within a target. Selection can then be followed by methods for improving the peptides and/or non-peptides to achieve better affinity and/or specificity. These diverse peptides and/or non-peptides may be conventional multi-chain antibodies (polyclonal or monoclonal), single-chain antibodies, or non-antibodies, including but not limited to peptides, products of phage display, aptamers, DNA, RNA, or modified DNA or RNA.

A well-known procedure for generation of large numbers of diverse peptides is through phage display, which is then followed by selection and can be further refined through other techniques such as molecular evolution (see, for example, Flores-Flores, C. et al, Development of human antibody fragments directed towards synaptic acetylcholinesterase using a semi-synthetic phage display library. J Neural Transm Suppl. 2002;(62):165-179; Qian, M.D, et al, Anti GPVI human antibodies neutralizing collagen-induced platelet aggregation isolated from a recombinant phage. Human Antibodies. 2002;11(3):97-105). scFv constructs can be made by linking variable domains of heavy (VH) and light (VL) chains together via a polypeptide linker (for example, see Asvadi P et al. Expression and functional analysis of recombinant scFv and diabody fragments with specificity for human RhD. J Mol Recognit 15:321-330, 2002). Peptides generated then selected (and then possibly improved) via this approach have been used in ELISAs and ELISA-like assays of their targets (e.g., see Schlattner U et al. Isoenzyme-directed selection and characterization of anti-creatine kinase single chain Fv antibodies from a human phage display library. Biochim Biophys Acta. 2002 Dec 12;1579(2-3):124-32; Oelschlaeger P et al. Fluorophor-linked immunosorbent assay: a time- and cost-saving method for the characterization of antibody fragments using a fusion protein of a single-chain antibody fragment and enhanced green fluorescent protein. Anal Biochem. 2002 Oct 1;309(1):27; Nathan S et al. Phage display of recombinant antibodies toward Burkholderia pseudomallei

exotoxin. J Biochem Mol Biol Biophys. 2002 Feb;6(1):45-53; Lu D et al. Fab-scFv fusion protein: an efficient approach to production of bispecific antibody fragments. J Immunol Methods. 2002 Sep 15;267(2):213-26; Zhang W et al. Production and characterization of human monoclonal anti-idiotype antibodies to anti-dsDNA antibodies. Lupus. 2002;11(6):362-9; Reiche N et al. Generation and characterization of human monoclonal scFv antibodies against Helicobacter pylori antigens. Infect Immun. 2002 Aug;70(8):4158-64; Rau D et al. Single-chain Fv antibody-alkaline phosphatase fusion proteins produced by one-step cloning as rapid detection tools for ELISA. J Immunoassay Immunochem. 2002;23(2):129-43; and Zhou B et al. Human antibodies against spores of the genus Bacillus: a model study for detection of and protection against anthrax and the bioterrorist threat. Proc Natl Acad Sci U S A. 2002 Apr 16;99(8):5241-6; Baek H et al., An improved helper phage system for efficient isolation of specific antibody molecules in phage display. Nucleic Acids Res. 2002 Mar 1; 30(5):e18).

scFv constructs can be based on antibodies, as in most of the references above, on T-cell receptors (e.g., Epel M et al. A functional recombinant single-chain T cell receptor fragment capable of selectively targeting antigen-presenting cells. Cancer Immunol Immunother. 2002 Dec;51(10):565-573), or on other sequences. Different phage coat proteins have been used to display the diverse peptides (see Gao C et al. A method for the generation of combinatorial antibody libraries using pIX phage display. Proc Natl Acad Sci USA. 2002 Oct 1;99(20):12612-6). For an example of fusion constructs, see Lu D et al. Fab-scFv fusion protein: an efficient approach to production of bispecific antibody fragments. J Immunol Methods. 2002 Sep 15;267(2):213-26.

For an example of molecular evolution to improve binding affinity, see Rau D et al. Cloning, functional expression and kinetic characterization of pesticide-selective Fab fragment variants derived by molecular evolution of variable antibody genes. Anal Bioanal Chem. 2002 Jan;372(2):261-7. Examples of other modifications "to improve affinity or avidity, respectively [include] by mutating crucial residues of complementarity determining regions or by increasing the number of binding sites making dimeric, trimeric or multimeric molecules." (the quote is from a review article, Pini A & Bracci L, Phage display of antibody fragments. Curr Protein Pept Sci. 2000 Sep;1(2):155-169). The initial set of diverse molecules can be enriched by using sequences from animals or humans exposed to or expressing antibodies against the target (see again Zhang W et al. Lupus 2002; and Reiche N et al. Infect Immun 2002).

Single chain antibodies can also be generated by using Escherichia coli (see Sinacola JR & Robinson AS, Rapid folding and polishing of single-chain antibodies from Escherichia coli inclusion bodies, Protein Expr Purif. 2002 Nov; 26(2):301-308.)

Non-antibodies capable of binding angiocidin fragments and/or aggregates of angiocidin also include aptamers and non-antibodies that comprise aptamers. Aptamers are DNA or RNA molecules that have been selected (e.g., from random pools) on the basis of their ability to bind to another molecule (discussed for example at the web site of the Ellington lab, in the Institute of Cellular and Molecular Biology, at the University of Texas at Austin, http://aptamer.icmb.utexas.edu/), wherein said molecule can be a nucleic acid, a small organic compound, or a protein, peptide, or modified peptide (such as angiocidin). An aptamer beacon is an example of a non-antibody that comprises an aptamer (See Hamaguchi N et al., Aptamer beacons for the direct detection of proteins. Anal. Biochem. 2001 Jul 15;294(2):126-131.)

An important aspect of the invention is the diagnostic use of immunoreactive angiocidin aggregates of angiocidin. These aggregates are useful in the detection of cancer using similar methods and applications described for the angiocidin fragments.

In one aspect, the invention includes a purified angiocidin fragment and/or aggregate that has been extracted from a bodily fluid, wherein the purified angiocidin fragment is immunoreactive with an antibody against angiocidin.

In another general aspect, the invention includes a method of producing antibodies and/or other binding agents against an angiocidin fragment and/or aggregate, the method comprising administering such a fragment and/or aggregate to an organism (especially a mammal or a bird) capable of producing antibodies. These fragments and/or aggregates include fragments and/or aggregates of different molecular weights. It is understood that said antibodies may comprise monoclonal antibodies and/or polyclonal antibodies. For monoclonal antibodies it is understood that cells from the organism are typically used in the production of hybridomas. For production of antibodies, including monoclonal antibodies, it is understood that any of the angiocidin fragments can be conjugated to a carrier molecule, including but not limited to keyhole limpet hemocyanin and bovine serum albumin, to facilitate the antibody response. A cell and a cell line for producing the aforementioned monoclonal antibodies are aspects of the invention. Examples of such cells include, but are not limited to, hybridomas, transfected cell lines, and infected cells. In one embodiment, said transfected cell lines are created by transfecting a cell line with an expression construct

or constructs directing the production of said antibodies. Said expression construct(s) and their sequences are also aspects of the invention.

The diagnostic assays of the instant invention can be used to detect angiocidin fragments and/or aggregates in various tumor types including but not limited to rectal carcinoma, pancreatic carcinoma, breast carcinoma, prostate carcinoma, melanoma, colon carcinoma and lung carcinoma.

In yet another aspect, the invention includes a method of producing a peptide binding agent or non-peptide binding agent against an angiocidin fragment or aggregate, or epitope therein, said method comprising the steps of 1) a generating step (random, semi-random, directed, combinatorial, and/or other) to generate large numbers (>100) of diverse peptides and/or non-peptides; 2) a selection step to identify within this large number those peptides and/or non-peptides that bind to the angiocidin fragment, aggregate, and/or an epitope therein; and 3) optionally an improvement step for improving the peptide or non-peptide binding agent to achieve better affinity and/or specificity.

In another aspect, the invention includes a cell line capable of producing a binding agent produced by the above described methods.

In another aspect, the invention includes a kit for the determination of the presence of, and/or the amount of, and/or the concentration of, an angiocidin fragment in a material taken or gathered from an organism, said kit comprising an angiocidin fragment. The invention also contemplates a kit for the determination of the presence of, and/or the amount of, and/or the concentration of, one or more angiocidin fragments and/or aggregates in a material taken or gathered from an organism, said kit comprising a binding agent capable of binding said one or more of said angiocidin fragments and/or aggregates and/or epitope therein.

In a particular aspect of the kit inventions, the angiocidin fragment is one that has been derivatized wherein the derivatization is selected from the group consisting of addition of a detectable label, incorporation of a detectable label, conjugation to another molecule, synthesis of the fragment as part of a chimeric protein, linkage to a carrier molecule or particle, linkage to a carrier, linkage to a bead, linkage to a solid matrix, linkage to keyhole limpet hemocyanin, linkage to an albumin, linkage to an ovalbumin, linkage to a cross-linking agent, linkage to an epitope tag, and linkage to an epitope.

Kits of the above inventions optionally comprise binding agents that distinguish between angiocidin and a fragment, between one fragment and another, between a fragment

and angiocidin, between a fragment and an aggregate, between one aggregate and another, and/or between angiocidin and an aggregate. If intended for solid tissue, the kits may comprise a homogenizing means for extracting a fragment into a solution, which optionally may also be provided.

Optionally, the kits comprise a means for separating or distinguishing a fragment or fragments or an aggregate from angiocidin. Examples of such means are a physical method to separate a fragment, angiocidin, and an aggregate, followed by detection; and a detection method that distinguishes a fragment, angiocidin, and an aggregate without requiring their physical separation beforehand. An example of the latter is a detection method that utilizes a binding agent that distinguishes among a fragment, angiocidin, and/or an aggregate.

The kits can also include an angiocidin fragment, a peptide derived from such fragment, and/or a derivatized fragment or peptide, to facilitate detection and calibration.

In one set of embodiments, the kits are adapted for use in an automated assay, such as one using a clinical autoanalyzer.

Particular kit aspects of the invention can also be summarized as follows:

A kit for the determination of the presence of, and/or the amount of, and/or the concentration of, a angiocidin fragment or fragments, or an aggregate or aggregates in a material taken or gathered from an organism, said kit comprising an angiocidin fragment or aggregate.

A kit for the determination of the presence of, and/or the amount of, and/or the concentration of, one or more angiocidin fragments or aggregates in a material taken or gathered from an organism, said kit comprising a binding agent capable of binding said one or more fragments or aggregates.

Particular embodiments are:

Such kits wherein the binding agent comprises a protein.

Such kits wherein said protein comprises an antibody.

Such kits wherein the antibody is a monoclonal antibody or a polyclonal antibody.

Such kits wherein said protein comprises a fragment of an antibody.

Such kits wherein said protein comprises a single-chain antibody.

Such kits wherein said single chain antibody is derived from a phage display library.

Such kits wherein said protein is a non-antibody, the non-antibody being a protein that is neither a multi-chain antibody nor a single-chain antibody.

Such kits wherein said protein non-antibody binding agent is selected from the group consisting of thrombospondin, a thrombospondin fragment, a CSVTCG-containing molecule, a binding agent that binds angiocidin, anti-secretory factor, and/or 26S proteasome non-ATPase regulatory subunit 4, fragments thereof that bind to their respective targets, and combinations, chimeras, and recombinant versions of said receptors and fragments.

Such kits wherein said binding agent comprises a non-protein.

Such kits wherein said binding agent comprises an aptamer.

Such kits wherein said binding agent recognizes angiocidin, anti-secretory factor, and/or 26S proteasome non-ATPase regulatory subunit 4.

Such kits wherein said non-antibody is selected from the group consisting of thrombospondin.

Such kits wherein said binding agent comprises an aptamer, meaning a DNA or RNA or related compound, that binds angiocidin, an angiocidin fragment or an aggregate.

Such kits wherein said binding agent comprises thrombospondin, a thrombospondin fragment, or a CSVTCG-containing protein.

The present inventions can also be summarized in the following manner:

One invention is a fragment-based diagnostic method for diagnosing, tracking a progression of, and/or determining the prognosis of a human or other mammal with a cancer said method comprising determining a level or presence of an angiocidin fragment (or an epitope present in an angiocidin fragment), wherein angiocidin has been defined as a molecule selected from the group consisting of a molecule whose amino acid sequence is SEQ ID NO:1, a molecule whose amino acid sequence is SEQ ID NO:2, and a molecule that binds to the CSVTCG peptide domain of thrombospondin and has a measured molecular weight of about 50 kD (i.e., 45 kD-55 kD) migrating in a single band when subjected to an SDS-PAGE (under non-reducing conditions).

Another invention is a fragment-based diagnostic method for diagnosing, tracking a progression of, and/or determining the prognosis of a human or other mammal with a cancer said method comprising determining a level or presence of an angiocidin fragment (or an epitope present in an angiocidin fragment), wherein angiocidin has been defined as a molecule selected from the group consisting of a molecule whose amino acid sequence is SEQ ID NO:1, a molecule whose amino acid sequence is SEQ ID NO:2, and a molecule that binds to the CSVTCG peptide domain of thrombospondin and has a measured molecular

weight of about 50 kD (45 kD-55 kD) migrating as a single band or about 60 kD (55 kD-65kD) migrating as a single band when subjected to an SDS-PAGE (under reducing conditions).

Another invention is a fragment-based diagnostic method further comprising comparing the level of said angiocidin fragment against known values for metastatic or nonmetastatic tumors.

Another invention is a fragment-based diagnostic method wherein the purified angiocidin fragment is not less than four amino acid residues in length and not more than 376 amino acid residues in length; alternatively, wherein the purified angiocidin fragment is not less than four amino acid residues in length and not more than 373 amino acid residues in length; althernatively, wherein the purified angiocidin fragment is not less than four amino acid residues in length and not more than 340 amino acid residues in length; alternatively, wherein the purified angiocidin fragment is not less than four amino acid residues in length and not more than 300 amino acid residues in length; alternatively, wherein the purified angiocidin fragment is not less than four amino acid residues in length and not more than 250 amino acid residues in length; alternatively, wherein the purified angiocidin fragment is not less than four amino acid residues in length and not more than 200 amino acid residues in length; alternatively, wherein the purified angiocidin fragment is not less than four amino acid residues in length and not more than 150 amino acid residues in length; alternatively, wherein the purified angiocidin fragment is not less than four amino acid residues in length and not more than 100 amino acid residues in length; alternatively, wherein the purified angiocidin fragment is not less than four amino acid residues in length and not more than 50 amino acid residues in length; and/or alternatively, wherein the purified angiocidin fragment is not less than four amino acid residues in length and not more than 25 amino acid residues in length. (The use of the word alternatively here and below is meant to describe specific embodiments of the invention.)

Another invention is a fragment-based diagnostic method wherein the level of the angiocidin fragment is analyzed from a sample of bodily fluid; alternatively, specifically wherein the bodily fluid is selected from the group consisting of blood, blood plasma, serum, lymph, cerebrospinal fluid, ascites fluid, urine, a lavage fluid, blister fluid, tears, saliva, a secretion, a mucous fluid, bile, milk, an aspirate, and cyst fluid' alternatively, specifically wherein the bodily fluid is blood; specifically wherein the bodily fluid is blood plasma; alternatively, specifically wherein the bodily fluid is serum; alternatively, specifically

wherein the level of angiocidin fragment is determined from a biopsy or other tissue sample and/or wherein analysis of the biopsy comprises a step selected from the group of immunohistochemical staining, immunofluorescent staining, immune staining, nonimmune staining; and an assay of the biopsy or an extract thereof.

Another invention is a fragment-based diagnostic method wherein analysis of the biopsy comprises an assay of a homogenated tissue or an extract thereof, and the level of the angiocidin fragment is normalized to a volume of sample and/or an amount of a protein and/or total protein and/or its level in a control or healthy sample; alternatively, specifically wherein the normalization comprises measurement of a total amount of a housekeeping molecule; and/or specifically wherein the housekeeping molecule is selected from the group consisting of actin, cyclophilin, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), 18S, and total protein.

Another invention is an aggregate-based diagnostic method of diagnosing, tracking a progression of, and/or determining the prognosis of a mammal with a cancer said method comprising determining a level or presence of an angiocidin aggregate, wherein angiocidin has been defined as a molecule selected from the group consisting of a molecule whose amino acid sequence is SEQ ID NO:1, a molecule whose amino acid sequence is SEQ ID NO:2, and a molecule that binds to the CSVTCG peptide domain of thrombospondin and has a measured molecular weight of about 50 kD when subjected to an SDS-PAGE under non-reducing conditions (and/or 50 kD or 60 kD when subjected to an SDS-PAGE under reducing conditions).

Another invention is an aggregate-based diagnostic method wherein the aggregate is more than 50 kD in apparent molecular weight on a non-reducing gel, and/or more than 60 kD in apparent molecular weight on a reducing gel; and/or specifically wherein the aggregate comprises a homodimer, heterodimer or a higher order complex; wherein the aggregate is formed by a process selected from the group consisting of self-aggregation, aggregation with another angiocidin molecule, aggregation of more than one angiocidin fragment, aggregation of an angiocidin fragment with a fragment of a molecule that is not angiocidin, aggregation of an angiocidin fragment with a molecule that is not a fragment, sulfydryl linkage with a second molecule or fragment, covalent linkage of angiocidin with another molecule or moiety, ionic linkage of angiocidin with another molecule or moiety, hydrophobic association of angiocidin with another molecule or moiety, homodimerization, heterodimerization and formation of a higher order complex; and/or specifically wherein the

level of the angiocidin aggregate is analyzed from a sample of bodily fluid; and/or specifically wherein the bodily fluid is selected from the group consisting of blood, blood plasma, serum, lymph, cerebrospinal fluid, ascites fluid, urine, a lavage fluid, blister fluid, tears, saliva, a secretion, a mucous fluid, bile, milk, an aspirate, and cyst fluid; specifically, wherein the bodily fluid is blood; and/or specifically wherein the bodily fluid is blood plasma; and/or specifically wherein the bodily fluid is serum; and/or specifically wherein the level of angiocidin aggregate is determined from a biopsy or other tissue sample; and/or specifically wherein analysis of the biopsy comprises a step selected from the group of immunohistochemical staining, immunofluorescent staining, immune staining, nonimmune staining, and an assay of the biopsy or an extract thereof.

Another invention is an aggregate-based diagnostic method wherein analysis of the biopsy comprises an assay of a homogenated tissue and the level of the angiocidin fragment is normalized to a volume of sample and/or an amount of a protein and/or total protein and/or its level in a control sample; and/or specifically wherein the normalization to a control sample comprises measurement of a total amount of a housekeeping molecule; and/or specifically wherein the housekeeping molecule is selected from the group consisting of actin, cyclophilin, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), 18S, and total protein.

The aggregate-based diagnostic method wherein the mammal is a human.

Another invention is a distinguishing diagnostic method that distinguishes two angiocidin fragments from each other, said fragments being a first fragment and a second fragment, respectively, said method comprising the steps of:

(1) utilizing an epitope or binding target shared by said first fragment and said second fragment as a target for a binding agent to obtain a quantification of a total of said first fragment plus said second fragment;

(2) utilizing an epitope or binding target present in said first fragment but not present in said second fragment, to obtain a quantification of said first fragment only; and

(3) utilizing the difference between the quantifications in steps (1) and (2) as a quantitation of the amount of said second fragment.

Another invention is a distinguishing diagnostic method that distinguishes two angiocidin fragments from each other, said fragments being a first fragment and a second fragment, respectively, said method comprising the steps of: utilizing an epitope or binding target present in said first fragment but not present in said second fragment, to obtain a

quantification of said first fragment only.

Another invention is a distinguishing diagnostic method wherein the method comprises a step wherein the fragment is physically separated from angiocidin, wherein angiocidin has been defined as a molecule selected from the group consisting of a molecule whose amino acid sequence is SEQ ID NO:1, a molecule whose amino acid sequence is SEQ ID NO:2, and a molecule that binds to the CSVTCG peptide domain of thrombospondin and has a measured molecular weight of about 50 kD when subjected to an SDS-PAGE.

Another invention is a distinguishing diagnostic method wherein the physical separation is accomplished using a technique that is selected from the group consisting of gel electrophoresis, dialysis, chromatography, size chromatography, affinity chromatography, CSVTCG affinity chromatography, immunoaffinity chromatography, adsorption, immunoadsorption, isoelectric focusing, mass spectrometry, centrifugation, sedimentation, floatation, precipitation, immunoprecipitation, extraction, and gel filtration.

Another invention is a distinguishing diagnostic method wherein the bodily fluid is selected from the group consisting of blood, blood plasma, serum, lymph, cerebrospinal fluid, ascites fluid, urine, a lavage fluid, blister fluid, tears, saliva, a secretion, a mucous fluid, bile, milk, an aspirate, and cyst fluid; specifically, wherein the bodily fluid is blood; specifically, wherein the bodily fluid is blood plasma; and/or specifically, wherein the bodily fluid is serum.

Another invention is a detection method for detecting a presence and/or a clinical course of a neoplastic disease by assaying a bodily fluid from an individual, wherein the method comprises the steps of:

(1) measuring the individual's bodily fluid level of an angiocidin fragment (and/or angiocidin fragment epitope);

(2) utilizing the result of step (1) in a diagnosis as to whether the individual has a neoplastic disease and/or whether a known neoplastic disease has progressed, regressed, or remained stable.

Another invention is a detection method wherein the bodily fluid is selected from the group consisting of blood, blood plasma, serum, lymph, cerebrospinal fluid, ascites fluid, urine, a lavage fluid, blister fluid, tears, saliva, a secretion, a mucous fluid, bile, milk, an aspirate, and cyst fluid; and/or specifically, wherein the bodily fluid is blood; and/or specifically, wherein the bodily fluid is blood plasma; and/or specifically, wherein the bodily

fluid is serum.

Another invention is a 2-individual detection method which is the detection method wherein the individual referred to therein is a first individual and wherein the method further comprises the steps of:

(3) measuring a second individual's level of the angiocidin fragment (and/or angiocidin fragment epitope) in the same type of bodily fluid utilized for step (1), said second individual considered to not have neoplastic disease; and

(4) utilizing the result of step (3) in the diagnosis of whether the first individual has a neoplastic disease.

Another invention is a 2-individual detection method wherein the first individual's level of an angiocidin fragment (and/or angiocidin fragment epitope) exceeds the level of the second individual, and this difference is used to conclude that it is more likely that the diagnosis will be that the first individual has a neoplastic disease and/or a neoplastic disease more advanced than that of the second individual.

Another invention is a 2-individual detection method further comprising the steps of assaying the individual's bodily fluid level for an angiocidin fragment (and/or angiocidin fragment epitope), and considering utilizing a change in bodily fluid level from an older to a more recent value to indicate appearance or progression or improvement, wherein said appearance or progression is indicated by an increase in the level of said angiocidin fragment and said improvement is indicated by a decrease in said level.

Another invention is a 2-individual detection method wherein the bodily fluid level of an angiocidin fragment (and/or angiocidin fragment epitope) is assayed on 2 or more days; and/or specifically wherein the bodily fluid level of an angiocidin fragment (and/or angiocidin fragment epitope) is assayed on 3 or more days spaced at regular intervals, said intervals ranging from two weeks to ten years; and/or specifically wherein the neoplastic disease is selected from the group consisting of an adenoma, an adenocarcinoma, a carcinoma, a lymphoma, a leukemia, a skin cancer, and a sarcoma; wherein the neoplastic disease is an internal cancer.

Another invention is a 2-individual detection method wherein the neoplastic disease is selected from the group consisting of a cancer of the respiratory system, a cancer of the circulatory system, a cancer of the musculoskeletal system, a cancer of a muscle, a cancer of a bone, a cancer of a joint, a cancer of a tendon and/or ligament, a cancer of a connective tissue, a cancer of the digestive system, a cancer of the liver and/or biliary system, a cancer

of the pancreas, a cancer of the head, a cancer of the neck, a cancer of the endocrine system, a cancer of the reproductive system, a cancer of the male reproductive system, a cancer of the female reproductive system, a cancer of the genitourinary system, a cancer of a kidney, a cancer of the urinary tract, a skin cancer, a cancer of another sensory organ, a cancer of the nervous system, a cancer of a lymphoid organ, a blood cancer, a cancer of a gland, a cancer of a mammary gland, a cancer of a prostate gland, a cancer of endometrial tissue, a cancer of mesodermal tissue, a cancer of ectodermal tissue, cancer of an endodermal tissue, a teratoma, a poorly-differentiated cancer, a well-differentiated cancer, and a moderately differentiated cancer.

Another invention is a 2-individual detection method wherein the neoplastic disease is selected from the group consisting of a cancer of solid tissue, a cancer of the blood or the lymphatic system, a solid cancer, a liquid cancer, a non-metastatic cancer, a premetastic cancer, a metastatic cancer, a cancer with vascular invasion, a skin cancer, a poorly differentiated cancer, a well-differentiated cancer and a moderately differentiated cancer; and/or specifically wherein the measurement of a level of an angiocidin fragment and/or aggregate and/or material containing an epitope that is present in an angiocidin fragment and/or aggregate comprises a use of a binding agent, said binding agent being capable of binding said fragment.

Another invention is a 2-individual detection method wherein said method comprises the use of a first binding agent, said first binding agent capable of binding angiocidin but not the angiocidin fragment further comprises a second binding agent, said binding agent capable of binding angiocidin and capable of binding the angiocidin fragment; and/or specifically, wherein said binding agent comprises a protein and/or a polypeptide; and/or specifically wherein said binding agent comprises an antibody or another molecule that crossreacts with or binds to an angiocidin fragment; and/or specifically wherein said antibody is selected from the group consisting of a monoclonal antibody a polyclonal antibody and single-chain antibody; and/or specifically wherein said protein comprises an antibody fragment; wherein said binding agent comprises a non-protein; wherein said protein and/or polypeptide is derived from a phage display library.

Another invention is a 2-individual detection method wherein the binding agent is selected from the group consisting of an antibody, an aptamer, a DNA, an RNA, a modified DNA, a modified RNA, a carbohydrate, a glycosaminoglycan, a heparin, a glycoprotein, a proteoglycan, and combinations and derivatives thereof; and/or specifically wherein said

binding agent comprises a ligand that binds an angiocidin fragment; wherein said ligand is selected from the group consisting of a thrombospondin, a thrombospondin fragment that binds angiocidin, a molecule comprising a thrombospondin fragment sequence that binds angiocidin, a molecule comprising the amino acid sequence CSVTCG (SEQ ID NO:3) and an antibody.

Another invention is a 2-individual detection method, wherein measuring the individual's bodily fluid level of an angiocidin fragment or fragments (and/or angiocidin fragment epitope) that is present in an angiocidin fragment further comprises the use of an angiocidin fragment as a standard.

Another invention is a 2-individual detection method wherein said angiocidin fragment used as a standard is selected from the group consisting of a recombinant angiocidin fragment, a purified angiocidin fragment that occurs in a mixture with other angiocidin fragments, a partially purified angiocidin fragment, and a synthetic angiocidin fragment.

Another invention is a 2-individual detection method wherein the angiocidin fragment is separated from angiocidin before said angiocidin fragment is bound to the binding agent, wherein angiocidin is a molecule selected from the group consisting of a molecule whose amino acid sequence is SEQ ID NO:1, a molecule whose amino acid sequence is SEQ ID NO:2, and a molecule that binds to the CSVTCG peptide domain of thrombospondin and has a measured molecular weight of about 50 kD when subjected to an SDS-PAGE under non-reducing conditions.

Another invention is a 2-individual detection method wherein said method comprises the use of a first binding agent, said first binding agent capable of binding angiocidin but not the angiocidin fragment further comprises a second binding agent, said binding agent capable of binding angiocidin and capable of binding the angiocidin fragment.

Another invention is a fluid-based, aggregate-based diagnostic method of detecting a presence and/or a clinical course of a neoplastic disease by assaying a bodily fluid from an individual, wherein the method comprises the steps of:

(1) measuring the individual's bodily fluid level of an angiocidin aggregate; and

(2) utilizing the result of step (1) in a diagnosis as to whether the individual has a neoplastic disease and/or whether a known neoplastic disease has progressed, regressed, or remained stable.

Another invention is a fluid-based, aggregate-based diagnostic method wherein the

bodily fluid is selected from the group consisting of blood, blood plasma, serum, lymph, cerebrospinal fluid, ascites fluid, urine, a lavage fluid, blister fluid, tears, saliva, a secretion, a mucous fluid, bile, milk, an aspirate, and cyst fluid; and/or specifically wherein the bodily fluid is blood; specifically, wherein the bodily fluid is blood plasma; and/or specifically, wherein the bodily fluid is serum.

Another invention is a fluid-based, aggregate-based 2-individual diagnostic method which is the fluid-based, aggregate-based diagnostic method wherein the individual referred to therein is a first individual and wherein the method further comprises the steps of:

(3) measuring a second individual's level of the angiocidin aggregate in the same type of bodily fluid utilized for step (1), said second individual considered to not have neoplastic disease; and

(4) utilizing the result of step (3) in the diagnosis of whether the first individual has a neoplastic disease.

Another invention is a fluid-based, aggregate-based 2-individual diagnostic method wherein the first individual's angiocidin aggregate level exceeds the angiocidin aggregate level of the second individual, and this difference is used to conclude that it is more likely that the diagnosis will be that the first individual has a neoplastic disease and/or a neoplastic disease more advanced than that of the second individual.

Another invention is a fluid-based, aggregate-based 2-individual diagnostic method further comprising the steps of assaying the individual's bodily fluid level for an angiocidin aggregate more than once, and considering utilizing a change in bodily fluid level from an older to a more recent value to indicate appearance or progression or improvement, wherein said appearance or progression is indicated by an increase in the level of said angiocidin aggregate and said improvement is indicated by a decrease in said level.

Another invention is a fluid-based, aggregate-based 2-individual diagnostic method wherein the bodily fluid level of an angiocidin aggregate is assayed on 2 or more days; and/or specifically wherein the bodily fluid level of an angiocidin aggregate is assayed on 3 or more days spaced at regular intervals, said intervals ranging from two weeks to ten years.

Another invention is a fluid-based, aggregate-based 2-individual diagnostic method wherein the neoplastic disease is selected from the group consisting of an adenoma, an adenocarcinoma, a carcinoma, a lymphoma, a leukemia, a skin cancer, and a sarcoma; and/or specifically wherein the neoplastic disease is an internal cancer.

Another invention is a fluid-based, aggregate-based 2-individual diagnostic method

wherein the neoplastic disease is selected from the group consisting of a cancer of the respiratory system, a cancer of the circulatory system, a cancer of the musculoskeletal system, a cancer of a muscle, a cancer of a bone, a cancer of a joint, a cancer of a tendon and/or ligament, a cancer of a connective tissue, a cancer of the digestive system, a cancer of the liver and/or biliary system, a cancer of the pancreas, a cancer of the head, a cancer of the neck, a cancer of the endocrine system, a cancer of the reproductive system, a cancer of the male reproductive system, a cancer of the female reproductive system, a cancer of the genitourinary system, a cancer of a kidney, a cancer of the urinary tract, a skin cancer, a cancer of another sensory organ, a cancer of the nervous system, a cancer of a lymphoid organ, a blood cancer, a cancer of a gland, a cancer of a mammary gland, a cancer of a prostate gland, a cancer of endometrial tissue, a cancer of mesodermal tissue, a cancer of ectodermal tissue, cancer of an endodermal tissue, a teratoma, a poorly-differentiated cancer, a well-differentiated cancer, and a moderately differentiated cancer.

Another invention is a fluid-based, aggregate-based 2-individual diagnostic method wherein the neoplastic disease is selected from the group consisting of a cancer of solid tissue, a cancer of the blood or the lymphatic system, a solid cancer, a liquid cancer, a non-metastatic cancer, a premetastic cancer, a metastatic cancer, a cancer with vascular invasion, a skin cancer, a poorly differentiated cancer, a well-differentiated cancer and a moderately differentiated cancer.

Another invention is a fluid-based, aggregate-based 2-individual diagnostic method wherein the measurement of an angiocidin aggregate level comprises a use of a binding agent, said binding agent being capable of binding said aggregate; and/or specifically wherein said method comprises the use of a first binding agent, said first binding agent capable of binding angiocidin but not the angiocidin aggregate, and further comprises a second binding agent, said binding agent capable of binding angiocidin and capable of binding the angiocidin aggregate, wherein angiocidin is a molecule comprising SEQ ID NO:1 or SEQ ID NO:2 and/or a molecule that binds thrombospondin through its CSVTCG peptide domain and has an apparent molecular weight of 50 kD in an SDS-PAGE gel under non-reducing conditions; and/or specifically wherein said binding agent comprises a protein and/or a polypeptide; and/or specifically wherein said binding agent comprises an antibody or another molecule that crossreacts with or binds to an angiocidin aggregate; said antibody is selected from the group consisting of a monoclonal antibody a polyclonal antibody and single-chain antibody; and/or specifically wherein said protein comprises an antibody

fragment; and/or specifically wherein said binding agent comprises a non-protein; wherein said protein and/or polypeptide is derived from a phage display library; and/or specifically wherein said binding agent is selected from the group consisting of an aptamer, a DNA, an RNA, a modified DNA, a modified RNA, a carbohydrate, a glycosaminoglycan, a heparin, a glycoprotein, a proteoglycan, and combinations and derivatives thereof; wherein said binding agent comprises a ligand that binds angiocidin or an angiocidin aggregate; and/or specifically wherein said ligand is selected from the group consisting of a thrombospondin, a thrombospondin fragment that binds angiocidin, a molecule comprising a thrombospondin fragment sequence that binds angiocidin, and a molecule comprising the amino acid sequence CSVTCG (SEQ ID NO:3).

Another invention is a fluid-based, aggregate-based 2-individual diagnostic method wherein said measuring the individual's bodily fluid level of an angiocidin aggregate further comprises the use of an angiocidin aggregate as a standard; and/or specifically wherein said angiocidin aggregate used as a standard is a purified angiocidin aggregate that occurs in a mixture with other angiocidin aggregates, and a partially purified angiocidin aggregate; wherein the angiocidin aggregate is separated from angiocidin before said angiocidin aggregate is bound to the binding agent.

Another invention is a polypeptide invention that is a polypeptide, the amino acid sequence of said polypeptide being one that is at least 4 amino acids in length and that is comprised of either a portion of SEQ ID NO:1 or a portion of SEQ ID NO:2, such that 1-10% of an N-terminus and/or 1-10% of a C-terminus of SEQ ID NO:1 is excluded from said portion of SEQ ID NO:1 and such that 1-10% of an N-terminus and/or a C-terminus of SEQ ID NO:2 is excluded from said portion of SEQ ID NO:2.

Another invention is a polypeptide invention that is a polypeptide, said polypeptide selected from the group consisting of (1) a polypeptide comprised of SEQ ID NO:1 provided that a portion of SEQ ID NO:1 is missing in the polypeptide, said missing portion selected from the group consisting of a signal peptide, a membrane association sequence, and a cell association sequence, and (2) a polypeptide comprised of SEQ ID NO:2 provided that a portion of SEQ ID NO:2 is missing in the polypeptide, said missing portion selected from the group consisting of a signal peptide, a membrane association sequence, and a cell association sequence.

Another invention is a polypeptide invention that is a polypeptide, the amino acid sequence of said polypeptide being one that is at least 4 amino acids in length and that is

comprised of either a portion of SEQ ID NO:1 or a portion of SEQ ID NO:2, such that 5-15% of the N-terminus and/or C-terminus of SEQ ID NO:1 is excluded from said portion of SEQ ID NO:1 and such that 5-15% of the N-terminus and/or C-terminus of SEQ ID NO:2 is excluded from said portion of SEQ ID NO:2.

Another invention is a polypeptide invention that is a polypeptide, the amino acid sequence of said polypeptide being one that is at least 4 amino acids in length and that is comprised of either a portion of SEQ ID NO:1 or a portion of SEQ ID NO:2, such that 10-25% of an N-terminus and/or a C-terminus of SEQ ID NO:1 is excluded from said portion of SEQ ID NO:1 and such that 10-25% of an N-terminus and/or a C-terminus of SEQ ID NO:2 is excluded from said portion of SEQ ID NO:2.

Another invention is a polypeptide invention that is a polypeptide, the amino acid sequence of said polypeptide being one that is at least 4 amino acids in length and that is comprised of either a portion of SEQ ID NO:1 or a portion of SEQ ID NO:2, such that 15-45% of an N-terminus and/or a C-terminus of SEQ ID NO:1 is excluded from said portion of SEQ ID NO:1 and such that 15-45% of an N-terminus and/or a C-terminus of SEQ ID NO:2 is excluded from said portion of SEQ ID NO:2.

Another invention is a purified angiocidin fragment that has been extracted from a bodily fluid, said fragment being one within a molecular weight range of 10 to 50 kD, wherein the size in kD is that determined by gel electrophoresis after disulfide bond reduction. In another embodiment, the molecular weight range is from 5-15 kD. In another embodiment, the molecular weight range is from 10-25 kD. In another embodiment, the molecular weight range is from 20-35 kD. In a further embodiment, the molecular weight range is from 30-45 kD.

Another invention is a purified angiocidin aggregate that has been extracted from bodily fluid, said aggregate being one with a molecular weight that is more than 60 kD, wherein the size in kD is that determined by gel electrophoresis after disulfide bond reduction.

Another invention is a purified angiocidin fragment that has been extracted from bodily fluid, said fragment being one with a molecular weight that is less than 50 kD, wherein the size in kD is that determined by gel electrophoresis under non-reducing conditions.

Another invention is a purified angiocidin aggregate that has been extracted from bodily fluid, said aggregate being one with a molecular weight that is more than 50 kD,

wherein the size in kD is that determined by gel electrophoresis under non-reducing conditions.Another invention is a method of producing antibodies against an angiocidin fragment or aggregate, said method comprising administering said fragment, aggregate or immunogenic portion thereof to an organism capable of producing antibodies; and/or specifically wherein polyclonal antibodies are produced; specifically, wherein monoclonal antibodies are produced; specifically, wherein antibodies produced are verified to bind an angiocidin fragment or aggregate. And another invention is an antibody produced by the method. A related invention is a cell line producing the monoclonal antibodies; and/or a DNA sequence producing a portion of the binding site of the monoclonal antibodies; said cell line is selected from the group consisting of a hybridoma, a transfected cell line, and an infected cell.

Another invention is a method of producing a peptide or non-peptide binding agent against an angiocidin fragment, or epitope therein, using methods known in the art for producing a binding agent. As an example, not meant to be restrictive, said method comprises the steps of

1) a generating step (random, semi-random, directed, combinatorial, and/or other) to generate large numbers (>100) of diverse peptides and/or non-peptides;

2) a selection step to identify within this large number those peptides and/or non-peptides that bind to the angiocidin fragment, angiocidin aggregate and/or an epitope therein; and

3) optionally an improvement step for improving the peptide or non-peptide binding agent to achieve better affinity and/or specificity. That method, wherein the binding agent is selected from the group consisting of a polyclonal antibody, a monoclonal antibody, a single-chain antibody, a non-antibody, a protein, a product of phage display, an aptamer, a DNA, an RNA, a modified DNA, a modified RNA, a carbohydrate, a glycosaminoglycan, a heparin, a glycoprotein, a proteoglycan, and combinations and derivatives thereof; and/or wherein the optional step for improving the binding agent is selected from the group consisting of molecular evolution, mutation of crucial residues, making dimeric, trimeric or multimeric molecules, and incorporation of sequences from animals or humans exposed to and/or expressing antibodies against the fragment or epitope therein; and/or wherein the initial set of diverse molecules is enriched by using sequences from animals or humans exposed to or expressing antibodies against the target. A related invention is a cell line capable of producing a binding agent produced by the method; and/or wherein said cell line

is selected from the group consisting of a hybridoma, a transfected cell line, and an infected cell.

Another invention is a method of diagnosing, tracking a progression of, and/or determining the prognosis of a human or other mammal with a cancer said method comprising reacting a sample from a patient with an antibody or other binding agent as to quantitate the total amount of reactive material, wherein said antibody or binding agent is reactive with an epitope or binding target present in angiocidin and/or angiocidin fragment and/or an angiocidin aggregate; and/or specifically wherein the epitope and/or binding agent is present in angiocidin; and/or specifically wherein the wherein the epitope or binding agent is present in an angiocidin fragment.

Another invention is a method that distinguishes two angiocidin fragments from each other, said fragments being a first fragment and a second fragment, respectively, said method comprising the steps of: utilizing an epitope or binding target present in said first fragment but not present in said second fragment, to obtain a quantification of said first fragment only.

Another invention is a method of generating an antibody or other binding agent reactive with an angiocidin fragment, said method comprising use of an angiocidin fragment as an immunogen. The angiocidin fragment can be purified or partially purified from a bodily sample, such as serum or plasma, from an individual with a cancer; or alternatively, it can be recombinant or synthetic but containing an epitope present in an angiocidin fragment in a bodily sample from an individual with a cancer. The fragment is used to immunize an animal; or as the target for phage display and/or aptamer generation; and so forth for other classes of binding agents, as described elsewhere in this application. Candidate binding agents are screened for their reactivity with an angiocidin fragment in a bodily sample, such as serum, from an individual or individuals with a cancer. The binding affinity of said binding agent can be improved through molecular evolution, mutation of crucial residues, making dimeric, trimeric or multimeric molecules, and incorporation of sequences from animals or humans exposed to or expressing antibodies against the fragment or epitope therein. For a binding agent that is an antibody, it can be selected from the group consisting of a monoclonal antibody, a polyclonal antibody, a recombinant antibody, and a single-chain antibody. The present invention also includes the antibody (or binding agent) itself, as well as its protein and/or DNA sequence. Methods that do not use a binding agent, such as mass spectrometry and/or sequencing, are also contemplated, for example, to detect the sequence

or sequence fragment of a motif that is present in an angiocidin fragment. Said sequence or sequence fragment may be present in other molecules of interest as well, such as angiocidin and/or an angiocidin aggregate.

Another invention is a method of generating a binding agent reactive with an angiocidin fragment, said method comprising the use of an angiocidin fragment.

Another invention is a method of diagnosing, tracking a progression of, and/or determining the prognosis of a human or other mammal with a disease or condition said method comprising determining a level or presence of an angiocidin fragment, wherein angiocidin is a molecule selected from the group consisting of a molecule whose amino acid sequence is SEQ ID NO:1, a molecule whose amino acid sequence is SEQ ID NO:2, and a molecule that binds to the CSVTCG peptide domain of thrombospondin and has a measured molecular weight of about 50 kD when subjected to an SDS-PAGE under non-reducing conditions; and/or alternatively wherein the disease or condition is a disease; and/or alternatively wherein the disease or condition is a condition.

Another invention is a binding agent, said binding agent capable of binding to an angiocidin fragment; and/or alternatively a polypeptide invention specified above.

Another invention is a binding agent that does not bind to angiocidin but binds to an aggregate that comprises angiocidin; and/or alternatively wherein the aggregate comprises angiocidin and further comprises non-angiocidin material.

In specific examples of the aforementioned inventions, fluid sampling techniques, volumes of fluids used in assays, details of antibodies or other binding agent reactions, and so forth will parallel those well established in the field of analogous diagnostic assays. Illustrative but not restrictive examples include assays for cytokines, growth factors, hormones, other proteins, and other molecules, which are available in ELISA plate format and in formats for clinical autoanalyzers. Commercial sources for such assays are well-known in the art and include but are not limited to Biosource (http://www.biosource.com/), Biomol (http://www.biomol.com/), Roche Diagnostics (http://www.roche-diagnostics.com/), and DPC-Biermann (http://www.dpc-biermann.de/immulite/main.htm). An illustrative summary of immunochemical methods in the clinical laboratory was prepared by Roger L. Bertholf, Ph.D., DABCC. Chief of Clinical Chemistry & Toxicology, UFHSC/Jacksonville and can be found at www.hscj.ufl.edu/path/lectures/Immunochemical_Methods.pps and is incorporated by reference herein. Several automated formats use bead-based assays, including but not limited to antibody-coated beads, avidin-coated beads, and magnetic

beads. It is understood that proteomic technologies can also be employed in the present invention, including but not limited to proteomic arrays.

While the invention has been described in detail and with reference to specific examples thereof, it will be apparent to one skilled in the art that various changes and modifications can be made therein without departing from the spirit and scope thereof.